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Changes in pyramidal and granular neuron numbers in the rat hippocampus 7 days after exposure to a continuous 900-MHz electromagnetic field during early and mid-adolescence

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ABSTRACT

The aim of this study was to investigate qualitative and quantitative changes in pyramidal and granule neurons in the male rat hippocampus after exposure to a continuous 900-megahertz (MHz) electromagnetic field (EMF) for 25 days during early and mid-adolescence. Three-week-old (21 day) healthy Sprague Dawley male rats were divided equally into control (CON), pseudo-exposed (PEX) and EMF groups. EMF rats were exposed to a 900-MHz EMF in an EMF-application cage, while the PEX rats were placed in the same cage without being exposed to EMF. No procedure was performed in CON. EMF was applied for 1 h/day, every day for 25 days. Following the 900-MHz EMF and pseudo-exposed applications, behavioral tests were performed for seven days. Then, all animals were euthanized and their brains were removed. Following histological tissue procedures, sections were taken from tissues and stained with toluidine blue. The optical fractionation technique was performed to estimate the pyramidal neuron numbers in the CA1, CA2-3 and hilus regions of the hippocampus and granule neuron numbers in the dentate gyrus region. Our findings indicated that the number of pyramidal and granule neurons in the hippocampus of the EMF group was statistically higher than PEX. Furthermore, the histopathological results showed that the cytoplasm of pyramidal (in the hilus, CA1, CA2 and CA3 region) and granular (in the dentate gyrus region) cells at the hippocampus were disrupted, as evident by intensive staining around cytoplasm and some artifacts were detected in the EMF group. In addition, statistical comparisons of the mean body weights and brain weights of the study groups revealed no significant differences. There was no statistically significant difference between the PEX and EMF groups in terms of temperature ($p > 0.05$) or humidity ($p > 0.05$) in the cages. In conclusion, higher numbers of both pyramidal and granule neurons were found in the male rat hippocampus after continuous 900-MHz EMF treatment.

1. Introduction

The effect of using devices such as radio, television and cell phones that emit an electromagnetic field (EMF) in daily life is continuously increasing in line with technological advances. Numerous studies have reported that EMF causes temporary pathological effects, while EMFs are associated with an increased risk for brain tumor development after use of mobile phones or cordless phones for 10 years (Hardell et al., 2007; ICNIRP, 2009; WHO, 2014; Morgan et al., 2015).

Cell phones are more widely used by the young and adolescent age groups (Independent Expert Group on Mobile Phones, 2000). This indicates that young people and adolescents are more exposed to the effect of EMF emitted by cell phones than adults. Adolescence in rodents lasts from postnatal days (PD) 21 to 60 and is subdivided into early adolescence (prepubescent animals, from PD 21 to 34), mid-adolescence (preadolescent, from PD 34 to 46) and late adolescence (young adult, from PD 46 to 59) (Tirelli et al., 2003). In our study, EMF was applied in early and mid-adolescence period. Cell phones generally operate at a frequency range of 900–1800 MHz (Dubreuil et al., 2002). Cell phones working at a frequency of 900-MHz are more common in Europe (Bas et al., 2009a; Dubreuil et al., 2002; Caplan et al., 2000). The male rats in our study were therefore exposed to the effect of a 900-MHz EMF. The effects of acute, chronic, continuous or interrupted application of EMF have been tested experimentally in numerous studies. However, no studies have been conducted in the early and middle adolescence period.

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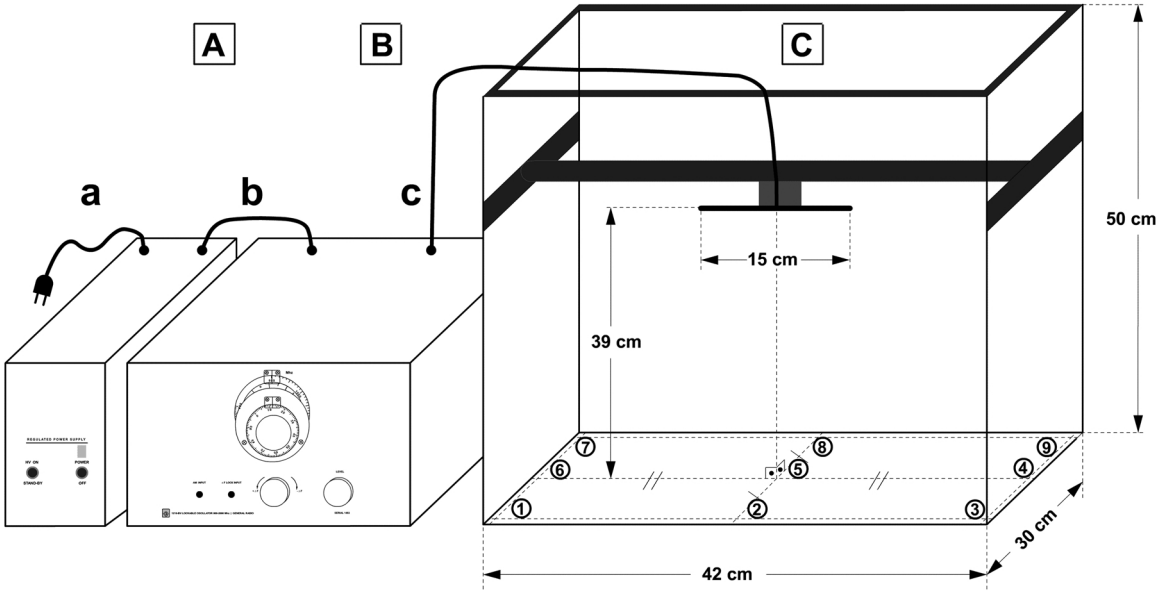

Fig. 1. The EMF application system, the dimensions of the EMF-application cage and the positions of the nine points on the floor of the cage at which EMF intensity was measured. (A) Uninterrupted regulated power source. (B) Ultra-high-frequency oscillator. (C) EMF-application cage. (a) Cable and plug transmitting electricity from the grid to the power source. (b) Cable connecting the power source and oscillator. (c) Coaxial cable between the oscillator and half-wave dipole antenna.

This study was therefore intended to examine the effects of EMF on rats in early and mid-adolescence. Exposure to EMF has been reported to cause several pathological effects. For instance dark staining in cell cytoplasm (Salford et al., 2003), reductions in the number of purkinje cells (Aslan et al., 2017) and pyramidal cells (Odaci et al., 2008; Bas et al., 2009a, b; Baş et al., 2013; Şahin et al., 2015), sleep disorders, fatigue, concentration disorders and warming of the ear (Durusoy et al., 2017), and changes in behavior, memory and learning (İkinci et al., 2013a). On the other hand, a previous study by Keleş et al. (2018) suggested that EMF has no effect on locomotor behavior, memory and learning. Another group reported that EMF strengthened the memory (Karimi et al., 2019). Research by Kim et al. (2006) showed that EMF exposure did not cause any alterations in urine analysis, blood analysis, blood biochemistry and tissues such as kidney, ovary, spleen, testis, liver, heart, lung and brain. Another study indicated that EMF caused oxidative stress in many tissues of the body (Kıvrak et al., 2017). Conversely, Budziosz et al. (2018) claimed that low-frequency electromagnetic field did not result in oxidative stress in the brain structures.

These results show that there is no consensus on the effects of exposure to EMF due to the widespread use of devices such as the cell phones, which are indispensable elements of daily life. Exposure to electromagnetic field may cause various types of effects on the nervous tissue, including reversible or irreversible damage. The exposure conditions such as the method, time, intensity, wave form, organ and age, is predicted to affect the study results (Hansson, 1988). Thus, it is important to employ methods in experimental studies that permit objective and reliable assessments and are not controversial among the scientific community. For this reason, we used the optical fractionator method to estimate neuron numbers in different regions of the hippocampus in male rats one week after exposure to 900-MHz EMF in early and mid-adolescence.

2. Materials and methods

2.1. Ethics statements

The Karadeniz Technical University (KTU) Animal Care and Ethics Committee approved the study (protocol number: 2013/67), and appropriate measures were taken by our study group to minimize animal pain or discomfort. The experimental part of this study was performed at the KTU Department of Histology and Embryology, Trabzon, Turkey, and stereological examination was performed at the Stereology and Electron Microscopy Laboratory, Centre for Stochastic Geometry and Advanced Bioimaging, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark. All experiments and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

2.2. Animals and experimental design

For this study, three-week-old healthy Sprague Dawley male rats were obtained from the KTU Experimental Animals Surgical Research and Application Center. These were then divided equally into CON, PEX and EMF groups. Throughout the study, rats were housed in standard plastic rat cages under normal laboratory conditions in rooms at a temperature of 22 ± 2 °C and relative humidity of 50% ± 5. Light was automatically adjusted to 12-h on and 12-h off. The animals were allowed ad libitum access to standard rat chow (Bayramoğlu Feed and Flour Industry Trading Corporation, Erzurum, Turkey) and tap water. Rats were weighed every five days during the experiment using a digital sensitive scale (KERN & SOHN GmbH, D- 72336 Balingen, Germany).

2.3. EMF-application system

An EMF-application system designed within the application room was used for PEX and EMF applications. This system was similar to the one used for 900-MHz EMF applications in previous studies (Odacı and Özyılmaz, 2015; İkinci et al., 2013a,b, 2016; Odacı et al., 2013, 2015, 2016; Odacı, 2017: Keleş et al., 2018). As described in detail in previous studies, the EMF-application system was installed in the application room. It consisted of an ultra-high-frequency oscillator (1218-BV, Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, Massachusetts, USA, Serial No. 1483) equipped with an uninterrupted power source (1267-B Regulated Power Supply, General Radio Company, Concord Massachusetts, USA, Serial No. 903), with an output power of approximately 300 mW and a frequency adjusted to 900-MHz. The oscillator was used to establish a 900-MHz EMF, approximately comparable to the frequency of cell phones. This was connected via a coaxial cable to a half-wave dipole antenna consisting of a copper rod

(1 mm ×15 cm). The dipole antenna was inserted in the central region, approximately 11 cm inside the open surface of the EMF-application cage. The EMF-application cage was designed to be sufficiently large to allow the rats to move around freely inside during applications. The cage was made of Plexiglas and measured 42 cm (length) ×30 cm (width) ×50 cm (height) in size (Şahin et al., 2015; Odacı, 2017) (Fig. 1).

2.4. Application procedures

PEX and EMF applications were performed in a different room from where the rats were housed. The application room which was designed only for EMF system administration was located within the same laboratory. The groups were placed in the system at different times. PEX rats were placed inside the EMF-application cage but were not exposed to the effect of EMF (the system was turned off). When the daily PEX application ended, the EMF rats were placed inside the EMF-application cage and exposed to the effect of a continuous 900-MHz EMF at the same time. Applications continued for each group (EMF and PEX) 1 h / day, every day for 25 days. No procedure was performed on CON rats. After the completion of the 25-day EMF/PEX application, behavioral tests were performed for seven days and after that the animals were sacrificed. Therefore, the tissues were harvested and analyzed seven days after the EMF exposure. The results of the behavioral tests were published elsewhere (Keleş et al., 2018).

EMF intensity was measured every day at various points, such as the corners, middle and walls of the application room using a broadband EMF measurement device (C.A 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France) with a range of 100 kHz-2.5 GHz. PEX and EMF applications were started once we had confirmed the absence of any other environmental EMF effect. The intensity of EMF was also measured using the same device every day during EMF application from nine different points on the floor and top of the EMF-application cage, both with rats inside the cage and with the cage empty (Fig. 2). Temperature and humidity levels in the EMF-application cage were measured every day during PEX and EMF applications with the help of a digital temperature and humidity measuring device (YCOM KMN-303, YuYau Shuanghe Electron Instrument Co. Ltd., Zhejing-China).

2.5. Histological preparation and section staining

At the end of the experiments, rats were euthanized by cervical dislocation. The brains were isolated from other tissues and weighed, and the right and left hemispheres were separated. Left brain hemisphere tissues were immersion-fixed in %10 formaldehyde solution. Tissues were fixed into molten paraffin by routine histological procedures [Washing under stream (1 night), 50% Alcohol (1 h), 70% Alcohol (1 h), 80% Alcohol (1 h), 96% Alcohol (1 h, 3 times repeated), Xylene (15 min, 3 times repeated), 60 °C Paraffin + xylene in the stove (1 h), paraffin in the stove (1 h), paraffin in the stove (1.5 h) followed by embedding into paraffin blocks].

Sections 30 µm in thickness were cut with the help of a microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany) in the cranial direction with a sampling rate of 1/5 systematic random method. The sections were placed in a 65 °C stove for 20–30 min and then stained with toluidine blue. A 1:4 mixture of water and 0.1% toluidine blue (Amplicon, AMPQY 3126, United States) was prepared for this purpose (Bancroft et al., 1994). Light microscope (Olympus, BX53, Japan) equipped with a digital camera (DP 80, Olympus, Japan) was used for histopathological evaluation.

2.6. Pre-study, hippocampal region and blind count

Pre-study was performed before the actual study, in which the step rate, dissector height, upper guard zone and unbiased counting frame measurements to be used during stereological analyses of 30-µm sections were determined. Previous studies were examined to differentiate the hippocampus regions. The hilus, dentate gyrus (DG), cornu ammonis 1 (CA1), cornu ammonis 2 (CA2) and cornu ammonis 3 (CA3) regions of the hippocampus were morphologically distinguished from one another by variations in cell sizes and shapes (Kaae et al., 2012; West et al., 1991).

Before the stereological analyses began, the sections to be analyzed

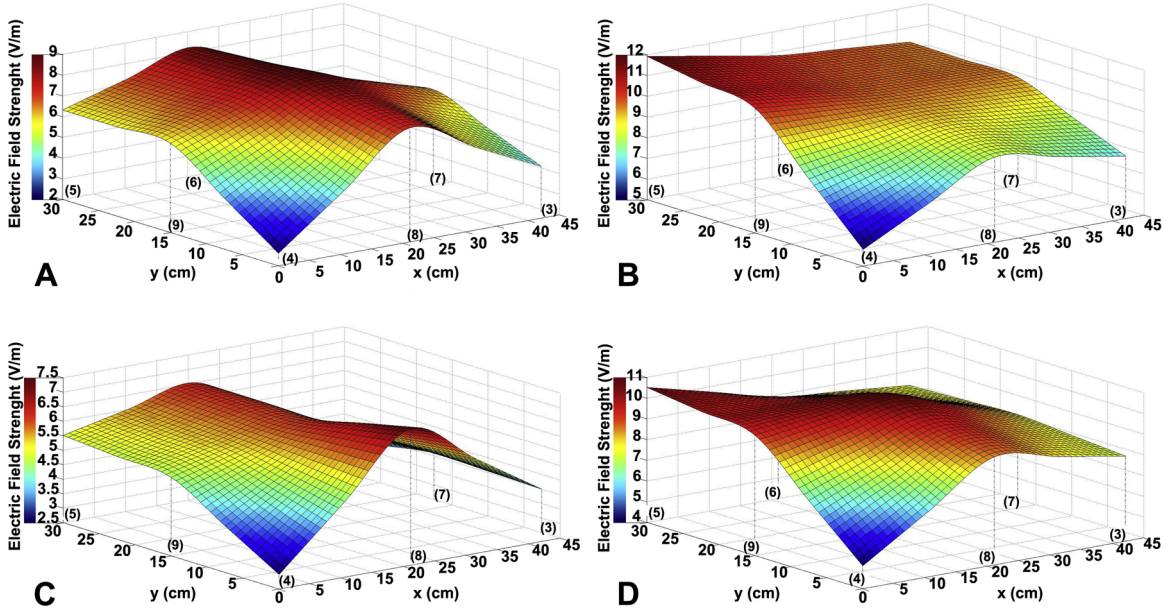

Fig. 2. Positional distribution on the x-y axes of mean EMF intensities measured from nine different points on the top and floor of the EMF-application cage during EMF application, with the cage empty and with rats inside. Positional distribution of EMF measurement results on the x-y axes on the floor of the EMF-application cage with the cage empty (A), positional distribution of EMF measurement results on the x-y axes on the top of the EMF-application cage with the cage empty (B), positional distribution of EMF measurement results on the x-y axes on the floor of the EMF-application cage with the cage full (C), and positional distribution of EMF measurement results on the x-y axes on the top of the EMF-application cage with the cage full (D).

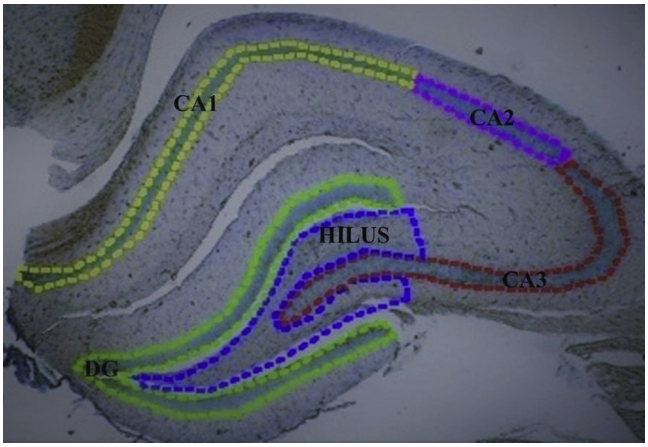

Fig. 3. Panoramic image of the hippocampus of the rat during stereology study (x2). Yellow region CA1, pink region CA2, red region CA3, blue region H, green region DG (C1, cornu ammonis 1; C2-3, cornu ammonis 2-3; DG, dentate gyrus; H, hilus).

were coded and any writing on them were concealed by researchers other than the author who would be performing the stereological analysis, and the sections were then given to that particular author. Thus, the researcher who performed the analyses was blinded about which section belonged to which animal group.

2.7. The stereological analysis system

Stereological analyses were performed on a computer (Fujitsu, Germany) equipped with stereological software and tools (newCAST, Visiopharm, Hørsholm, Denmark), two monitors (DELL, USA), a light microscope (Olympus BX50, Olympus, Denmark), a digital camera modified for stereological analyses attached to the microscope (PixeLINK PLA686C, Canada), a motorized deck allowing movements on the *X* and *Y* axes to be controlled from the computer (Prior H138 Cambridge, UK), an automatic deck (Prior H138BX, Cambridge, UK), a control panel (Prior H29, Cambridge, UK) and a microcator display (Heidenhain D-83301, Germany).

2.8. Details of stereological analyses

The microscope was set at the smallest lens magnification (2× / 0.08), and the various sub-regions of the hippocampus were marked using different colors (Fig. 3). The microscopic magnification was then adjusted to permit neuron counting (60× / 1.35) (Fig. 4–6). The step interval, optic dissector height, upper guard zone and counting frame area were logged into the system (Table 1). The sampling performed for each region was applied to all sections in the same manner. Total neuron numbers in the hilus, DG, CA1, and CA2/CA3 regions of the hippocampus were estimated for each animal. Stereological analyses were performed using sections obtained with sampling from four different regions of the hippocampus from each group in order to elicit neuron numbers.

2.9. Body and brain weight analyses

Body weight increase in relation to time was monitored via weight measurements which were performed every five days in rats belonging to different groups since the start of the study (Fig.7)

2.10. EMF-cage humidity and temperature analyses

The mean internal temperature in the cage during the 25 days in which EMF was applied was 21.8 ± 2.0 °C, while the mean humidity level was 37.8 ± 2.9 g/m^3. Mean temperature during PEX application over the 25 days of study was 22.1 ± 1.9 °C, while the mean humidity was 38 ± 2 g/m^3 (Fig. 8).

2.11. Statistical analyses

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, version 13.1, SSPS Inc., Chicago, IL, USA) software. Kruskal Wallis analysis of variance was used to compare differences between group parameters. Dual comparisons between groups exhibiting significant values were evaluated using the Mann–Whitney U-test with the corrected Bonferroni test. Results are expressed as mean ± standard deviation (mean ± SD). p-values less than 0.05 was regarded as statistically significant for all comparisons.

3. Results

3.1. Body and brain weight findings

Statistical comparisons of the mean body weights of the study groups revealed no significant differences between CON and EMF, or PEX and EMF ($p = 0.732$; $p = 1.000$, respectively). Mean body weight values obtained every five days were 111 ± 52.6 g for CON, 118.5 ± 57.5 g for PEX and 118.7 ± 57.8 g for EMF. Mean body weight values obtained at 32nd day were 191,7 ± 26,5 g for CON, 198,7 ± 10,8 g for PEX and 181,7 ± 13,2 g for EMF. Additionally, no significant variation was observed at statistical analyses ($p > 0.05$). Mean brain weights of the rats from CON, PEX and EMF were 1.2 ± 0.05 g, 1.3 ± 0.6 g and 1.3 ± 0.09 g, respectively. Statistical comparison of brain weights revealed no significant differences between CON and EMF, or PEX and EMF ($p = 0.109$; $p = 0.643$, respectively).

3.2. EMF-cage humidity and temperature results

Statistical analysis of these findings revealed no significant difference between the PEX and EMF in terms of temperature ($p > 0.05$) or humidity ($p > 0.05$) (Fig. 8).

3.3. Histopathological results

The results of histopathological evaluation of this study were previously published in another paper by Keleş et al. (2018). The hippocampus regions of the control and sham group were normal (Figs. 4 and 5). In the EMF group, the cytoplasm of the pyramidal cells and granular cells was observed to be disrupted. Also, intensive staining around cytoplasm and some artifacts were detected (Fig. 6).

3.4. EMF measurements

On the basis of EMF measurements taken every day throughout the study with the empty EMF-application cage, mean positional EMF intensity was found to be 6.37 V/m on the bottom surface of the EMF-application cage and 11.46 V/m on the top. Values with rats inside the EMF-application cage were 6.24 V/m on the bottom surface of the EMF-application cage and 9.55 V/m on the top. These measurements showed that EMF rats were exposed to a mean EMF intensity of 9.55 V/m in the EMF-application cage (0.242 W/m^2) (Fig. 2). The specific absorption rate (SAR) was calculated at 0.012 W/kg on the basis of these measurements (RadHaz SAR Equivalence Calculator Version 1.0, © 2000 Richard Tell Associates, Inc., Mesquite, NV, USA) (Baş et al., 2013; İkinci et al., 2013a; Odacı et al., 2013, 2015).

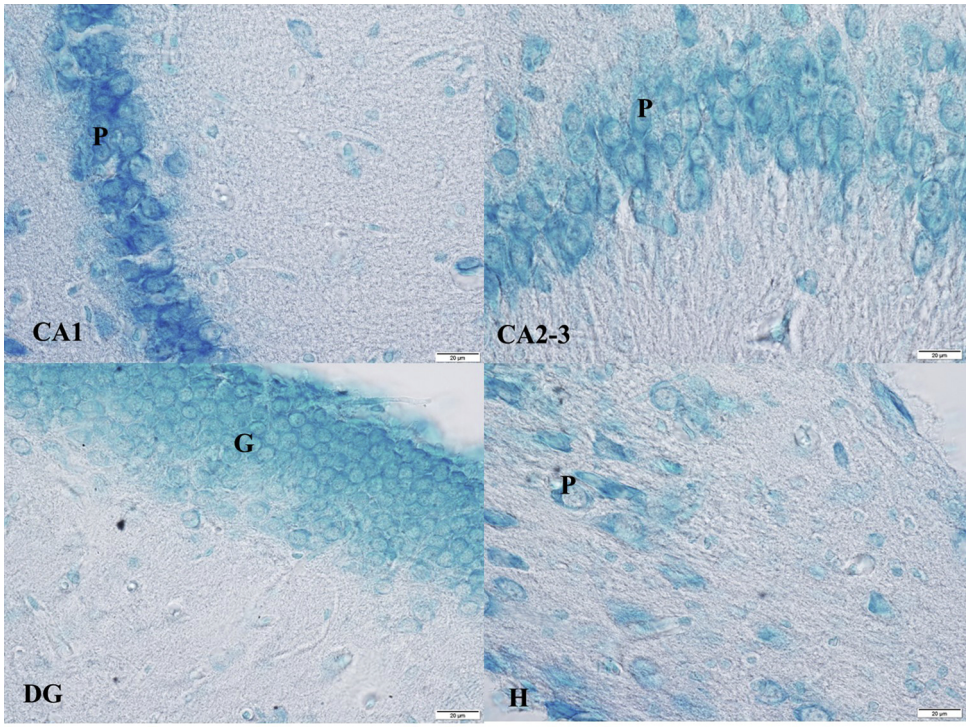

Fig. 4. Panoramic image of the hippocampus cells of the control group rats is shown (x60). Pyramidal neurons (P) in the C1, C2-3 and H regions of the hippocampus and granular neurons (G) in the DG region (C1, cornu ammonis 1; C2-3, cornu ammonis 2-3; DG, dentate gyrus; H, hilus).

3.5. Neuron data for hippocampal regions

3.5.1. Pyramidal neuron numbers in the hilus region

Stereological analysis revealed no significant difference in terms of total numbers of pyramidal neurons in the hippocampal hilus between EMF and CON ($p = 0.72$), while the number of pyramidal neurons in EMF was higher than in PEX ($p = 0.040$). No statistically significant difference was observed in terms of total pyramidal neuron numbers between CON and PEX ($p = 0.259$) (Table 2).

3.5.2. Granule neuron numbers in the dentate gyrus region

Stereological analysis of the hippocampal DG region revealed a

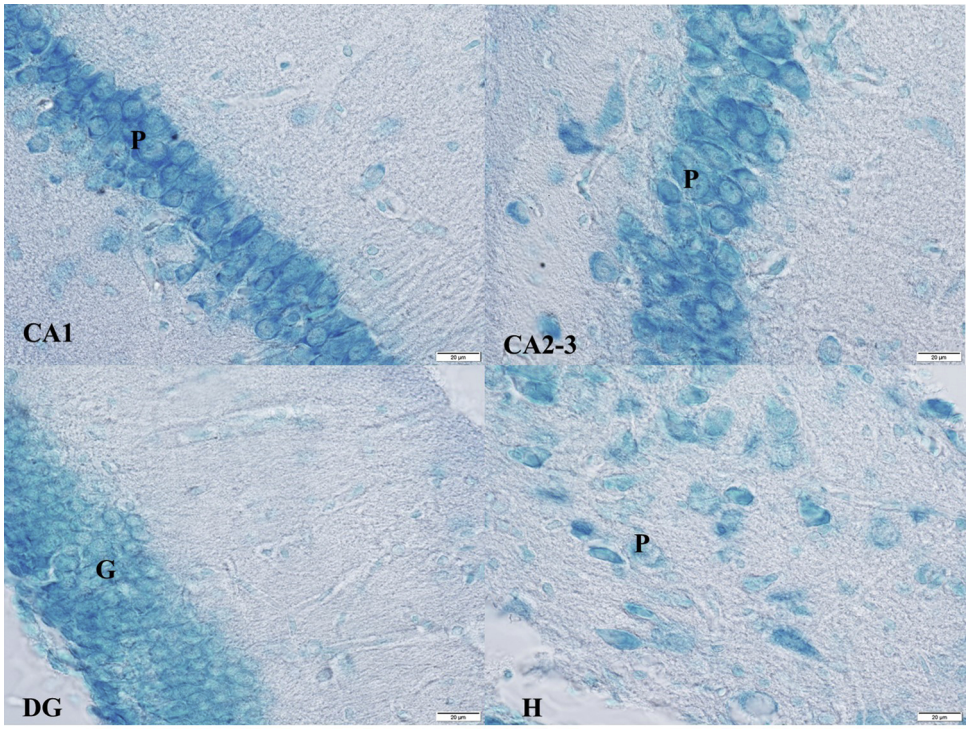

Fig. 5. Panoramic image of the hippocampus cells of the sham group rats is shown (x60). Pyramidal neurons (P) in the C1, C2-3 and H regions of the hippocampus and granular neurons (G) in the DG region (C1, cornu ammonis 1; C2-3, cornu ammonis 2-3; Dg, dentate gyrus; H, hilus).

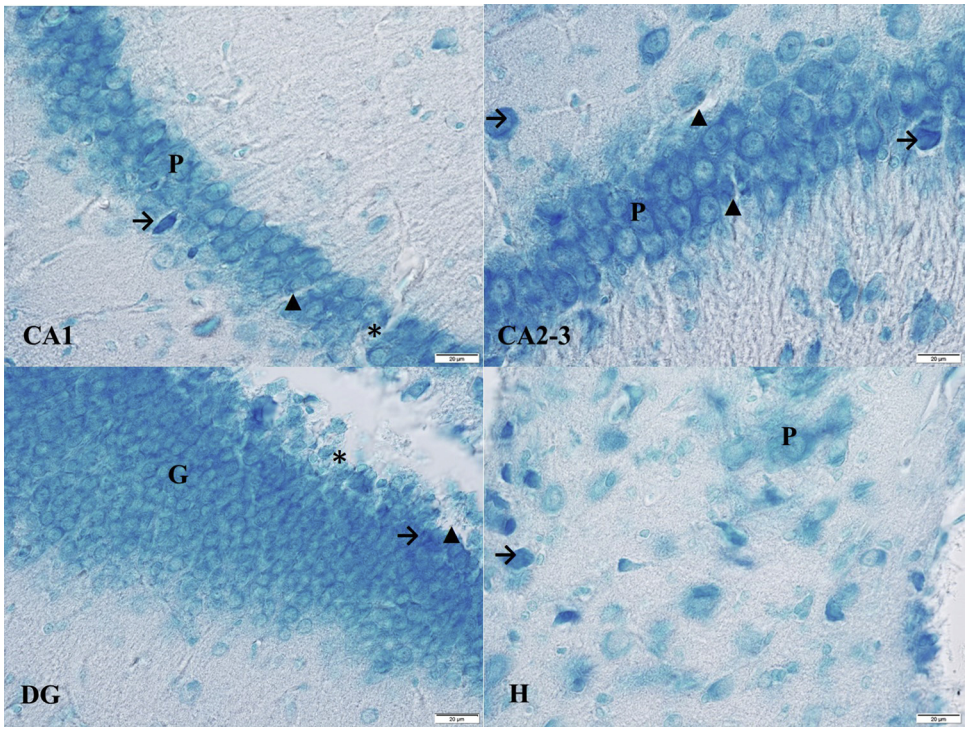

Fig. 6. Panoramic image of the hippocampus cells of the EMF group rats is shown (x60). Pyramidal neurons (P) in the C1, C2-3 and H regions of the hippocampus and granular neurons (G) in the DG region. Histological changes are observed impairment of neuron cytoplasm (▲), dark staining in cytoplasm (→) and artifact (*). (C1, cornu ammonis 1; C2-3, cornu ammonis 2-3; DG, dentate gyrus; H, hilus).

Table 1
The data used during stereological analysis.

Parameters	Hippocampus regions			
	Dentate gyrus	Hilus	CA1	CA2-3
Section sampling ratio	1/5	1/5	1/5	1/5
Unbiased counting frame area (μm^2)	240	1200	650	650
Disector height (μm)	10	10	10	10
Upper guard zone (μm)	5	5	5	5
Step interval (μm)	150	150	150	150
Coefficient of error	0.06	0.11	0.06	0.07
Mean section thickness (mean ± SD	20.86 ± 0.05			

statistically significant difference between EMF and PEX (p = 0.021) in terms of total granule neuron numbers. The number of granule neurons in EMF was higher than in PEX. No statistically significant difference in terms of total granule neuron numbers was observed between CON and EMF or CON and PEX (p = 0.72; p = 0.535, respectively) (Table 2).

3.5.3. Pyramidal neuron numbers in the cornu ammonis 1 region

Analysis of the CA1 region of the hippocampus revealed statistically significant differences in terms of total pyramidal neuron numbers between EMF and PEX and between EMF and CON (p = 0.040; p = 0.021, respectively). The number of pyramidal neurons was also higher in EMF compared to the other two groups. No statistically significant difference was determined between CON and PEX (p = 0.805) (Table 2).

3.5.4. Pyramidal neuron numbers in the cornu ammonis 2-3 region

Stereological analysis of the CA2-3 region of the hippocampus revealed a statistically significant difference in terms of total pyramidal neuron numbers between CON and EMF (p = 0.029), and the number of pyramidal neurons in EMF was higher than in CON. There was no

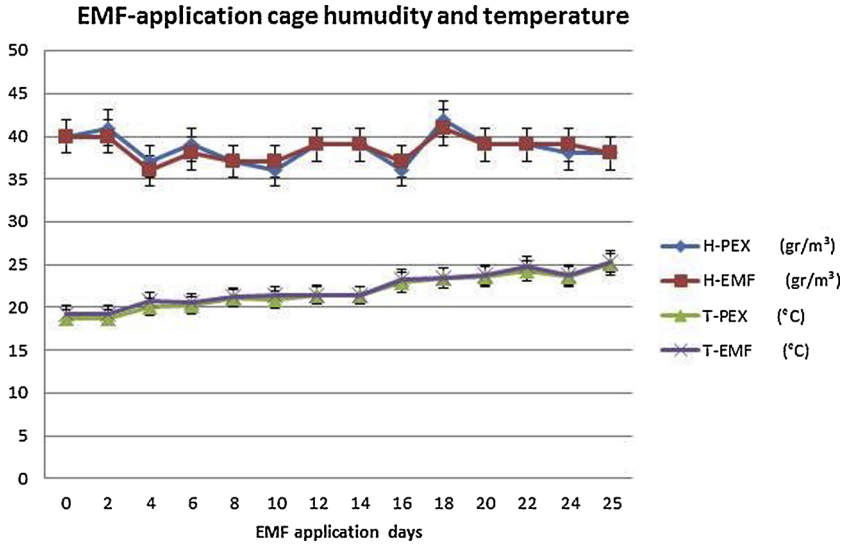

Fig. 7. Increases in body weights of rats from the different groups during EMF application.

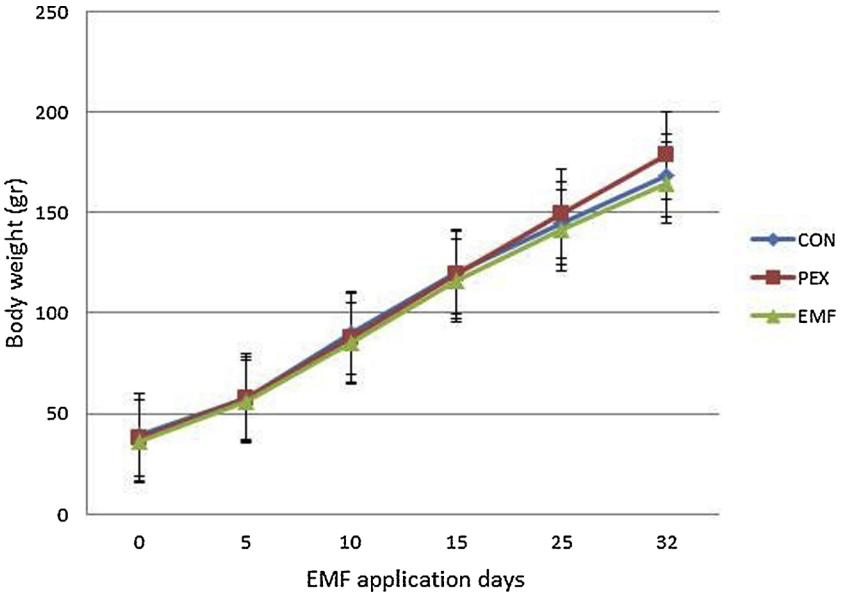

Fig. 8. Changes in temperature and humidity in the EMF-application cage during application in the pseudo-exposed and EMF groups during application (H-PEX = pseudo-exposed group humidity; H-EMF = EMF group humidity; T-PEX = pseudo-exposed group temperature; T-EMF = EMF group temperature).

Table 2
Mean total neuron numbers in the hilus, dentate gyrus, CA1 and CA2-3 regions of the hippocampus of CON, PEX and EMF rats.

Groups	Hippocampus regions			
	Dentate gyrus (Granule neurons)	Hilus (Pyramidal neurons)	CA1 (Pyramidal neurons)	CA2-3 (Pyramidal neurons)
CON	1014876.4 ± 33313.2	37060.2 ± 1102.6	197736.1 ± 8328.2	143512.3 ± 7804.2
PEX	966972.5 ± 33860.9	34664.4 ± 1574.8	189184.9 ± 18783.9	147386.2 ± 19051.5
EMF	1114276.4 [a] ± 56787	42176.3 [b] ± 2780.6	249895.2 [c] ± 15265.5	188324.8 [d] ± 17567.1

Data represent mean ± SD, n = 7 for each group; CA, cornu ammonis; CON, control group; PEX, pseudo-exposed group; EMF, EMF group.
[a] Total granule neuron numbers in EMF in the DG region are statistically significantly higher compared to PEX ($p = 0.021$).
[b] Total pyramidal neuron numbers in EMF in the hilus are statistically significantly higher compared to PEX ($p = 0.040$).
[c] Total pyramidal neuron numbers in EMF in the CA1 region are statistically significantly higher compared to CON and PEX ($p = 0.021$ and $p = 0.040$, respectively).
[d] Total pyramidal neuron numbers in EMF in the CA2-3 region are statistically significantly higher compared to CON ($p = 0.029$).

statistically significant difference between CON and PEX ($p = 0.902$) or PEX and EMF ($p = 0.189$) (Table 2).

4. Discussion

Studies have reported a decrease in numbers of pyramidal neurons in the hippocampus (Şahin et al., 2015; Baş et al., 2013; Bas et al., 2009a, b) and of granule neurons in the DG region of the hippocampus (Odaci et al., 2008) following application of EMF in the prenatal and postnatal periods. Significantly higher neuron numbers were observed in the present study in the EMF CA1 and CA2-3 regions compared to CON. Similarly, neuron numbers in the hilus, DG and CA1 regions were significantly higher than in PEX. On the basis of these results it is possible to suggest that exposure of male rats to the effect of a 900-MHz EMF on postnatal days 21–46 (early and mid-adolescence) causes an increase in cell numbers in the hippocampus one week after EMF exposure. No similar findings have been described in the literature to date, and this is the first such report.

Cells with abnormal shapes and morphological structures have been reported in studies investigating the effects of EMF on the rat hippocampus (Salford et al., 2003; Bas et al., 2009b). Similarly, part of this study consisted of histopathological evaluations, which reported darkly stained neurons between normal pyramidal neurons in the CA and DG region of hippocampus (Kele et al., 2018). These and other previous studies show that some neurons exhibit injury following EMF application and lose vitality as their normal shapes are compromised. In other words, in light of these findings in the literature, we expected a decreased number of neurons in the EMF group. However, despite all these previous reports, we counted a higher number of pyramidal and granule cells in the hippocampus in our study.

Considering the higher number of neurons that we detected, it may be concluded that damage occurring at the cellular level under the effect of 900-MHz EMF persisted even one week after discontinuation of the EMF application while the neuron numbers increased during this process, both as a result of initiation of cell proliferation and the replacement of damaged neurons under the effect of EMF by new ones (Baş et al., 2013; Odaci et al., 2008; Bas et al., 2009a,b). We think that the one-week period between EMF application and the rats being euthanized is significant in this variation in neuron numbers, because in studies reporting a decrease in neuron numbers following EMF application, rats were euthanized immediately after the application of EMF (the same day or one day later) (such as Bas et al., 2009b). In our study, however, neuron numbers in the hippocampus were investigated with rats being euthanized for removal of brain tissue one week after the end of EMF application due to behavioral tests (Keleş et al., 2018). Various reasons may be hypothesized for the higher neuron numbers observed during this one-week period. One possibility is that the neuronal stem cells (NSCs) could have been activated under the effect of 900-MHz EMF (de Miranda et al., 2017).

As published in earlier studies, NSCs in the rat brain are found both

in the subgranule zone of the hippocampal DG and the subventricular zone of the lateral ventricles (Ming and Song, 2011; Gage, 2000; Kuhn et al., 1996; Cameron et al., 1993). Studies have reported that various agents initiate neurogenesis in the rat hippocampus. Also, microglia, astrocytes and neuron levels affect neurogenesis (de Miranda et al., 2017). This and similar studies suggest that different drugs or environmental factors can initiate neurogenesis in the rat hippocampus and can cause cell proliferation and an increase in neuron numbers (Leone et al., 2015; Malberg et al., 2000). Neurogenesis could explain the increased number of cells in the DG. But it fails to explain the cause of the increased neuron number in the rest of the hippocampus. The cause of increased cell numbers within the CA1 and CA2 regions is unknown. Chronic EMF may inhibit neuronal death in the CA1 and CA3. Significantly higher neuron numbers were observed in the present study in the EMF CA1 and CA2-3 regions compared to CON. On the basis of our study findings, we may conclude that 900-MHz EMF is one such agent.

In our study, EMF granular cell increase was observed in DG region. Research has shown that cell proliferation in the dentate gyrus of the rat hippocampus is characteristically suppressed by soft diet feeding (Aoki et al., 2005). Other researchers reported that epilepsy (Varodayan et al., 2009), running (Praag et al., 1999), antidepression treatment (Malberg et al., 2000) etc. increases cell proliferation in the dentate gyrus. As a result of our study, it can be suggested that the EMF causes increased granule cell numbers in the DG region.

On the other hand, various studies involving extremely low-frequency electromagnetic field (ELF-EMF) have reported that this can initiate neurogenesis in the mouse or rat hippocampus (Leone et al., 2015; Di Lazzaro et al., 2013; Podda et al., 2013; Piacentini et al., 2008). Leone et al. (2014) reported that mice exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) showed increased hippocampal neurogenesis. They also suggested that ELF-EMF induced proneuronal differentiation of gene expression in hippocampal neural stem cells. Therefore, when other studies involving ELF-EMF stimulation are considered, we think that the hypothesis proposed on the base of our study and also suggested in other research may present clinical importance in the treatment of some neurodegenerative diseases that manifest with neuronal loss (such as Alzheimer's). However, the answer to the question of whether new cells forming as a result of proliferation due to the effect of 900-MHz EMF replace those that are damaged by the EMF application is particularly important here in terms of both physiological and morphological characteristics.

Another cause of cell growth may be brain tumour. Some researchers have reported that long-term use of cell phones is associated with increased brain tumour incidence (Hardell et al., 2007; ICNIRP, 2009; WHO, 2014; Morgan et al., 2015). On the contrary, Karipidis et al. (2018) claimed that use of cell phones did not result in an increase in any histological type of brain tumors or affect their locations. Additionally, in this study, behavior tests were performed in the last adolescent period. In these test results, no effect of EMF on behavior and learning was observed (Kele et al., 2018). These results can show us that there is no damage to the cells. However, different methods should be applied to reach a correct result.

To put it another way, the questions of whether the cells that proliferate due to the effect of 900-MHz exhibit pathological characteristics and can lead to malignant pathologies need to be answered by means of further studies employing different methods. Even if the results of such experimental studies are not also observed in humans, the findings from studies involving rodents such as rats can still elicit important information in terms of human health. The findings of many studies involving rodents can be compared with humans at an equivalent stage of development, irrespective of whether they are pre- or postnatal (Dobbing, 1970; Dobbing and Sands, 1973; Rodier, 1980; Jacobson, 1991; Odaci et al., 2008). The study results can therefore be compared with the adolescent human brain, in which the development has not yet been completed. In conclusion, our results show that continuous 900-MHz EMF application for one hour per day in early and mid-adolescence for 25 days leads to higher numbers of both pyramidal and granule neurons in the male rat hippocampus.

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